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Supercritical Fluid Extraction of *Swietenia mahagoni* Seed: Antioxidant and Antimicrobial Activities

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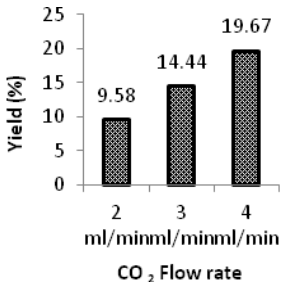
Graphical abstract



Abstract

Swietenia mahagoni seeds have been used as folk medicine for a treatment of hypertension, malaria and diabetes. This research was conducted to obtain extracts from the *Swietenia mahagoni* using supercritical fluid extraction (SFE) with pure CO_2 solvent, in order to evaluate the high- pressure method in terms of process yield and biological activity. The various conditions namely flow rate of CO_2 were set up at 2, 3, and 4 ml/min; at constant pressures (P) and temperature (T). The sample extracts obtained by SFE with CO_2 flow rate of 4 ml/min showed the highest percentage yield (19.67%) compared to the others. The antioxidant potential of the extracts was evaluated by the DPPH method and Folin- Ciocalteu method. The solvent flow rate of 2 ml/min gave the lowest percentage of yield, but good results in antioxidant activity and total phenolic content. The antimicrobial activity of the extracts against Gram-positive (*Bacillus subtilis, Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) was evaluated based on the inhibition zone using disc diffusion assay. These results ensured that *Swietenia mahagoni* seed extract had inhibitory effects on the growth of *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* at concentration of 100 mg/ml.

Keywords: Supercritical fluid extraction (SFE); Swietenia mahagoni; antioxidant; antimicrobial

1.0 INTRODUCTION

Swietenia mahagoni seeds have been applied as folk medicine for treatment of hypertension, malaria, and diabetes [1]. The therapeutic effects associated with the seeds are mainly caused by the biologically active ingredients, fatty acids and tetranortriterpenoids [2]. There are reports of *S. mahagoni* seeds having anti-inflammatory, antimutagenecity, and antitumor activities [3]. The plant extracts have been accounted to possess antibacterial and antifungal activities. Therefore, in the last several years, studies regarding extraction of natural compounds, capable to avoid microorganism's development, present special interest, especially related to microorganisms responsible for the deterioration of foods and ones responsible for a great number of diseases [4].

Supercritical fluid extraction (SFE) is based on the use of solvents in conditions above the critical point, resulting in a liquid like density, gas like viscosity and the diffusivity values within two orders of magnitude higher than that for typical liquids. This technology presents several advantages over traditional liquid-solvent-based methods including improved selectivity, expeditiousness, automation and environmental safety as the solvent can easily be removed from the solutes by expansion to ambient pressure [5]. Carbon dioxide (CO_2) is commonly used in SFE because, in contrast with organic solvents, it is non-toxic, inexpensive and volatile, with moderate critical conditions, thus no thermal or chemical degradations of bioactive substance are expected [6]. The quality and the composition of the extracts are strongly dependant on the extraction technique, the solvent used, the origin of the raw material, the part of the plant used (skin, seeds, leaves, etc), its storage condition and the pre-treatment applied [7,8]. The quality of the extract can be represented by its properties, such as its biological activities. In this study, the extract yield, antioxidant and antimicrobial activities of the extracts from *S. mahagoni* seed obtained by SFE with pure CO_2 of different flow rate.

■2.0 MATERIAL AND METHODS

2.1 Raw Material and Sample Preparation

The *S. mahagoni* seeds were collected from Indonesia. Then, the seeds were rinsed with tap water to remove any foreign particles and dirt prior to drying. Then, the cleaned seeds were cut into small pieces and dried by using oven at temperature of 50°C for one week to remove moisture. The seeds were powdered by using a blender (Merck Panasonic).

2.2 Supercritical Fluid Extraction (SFE)

Extraction was conducted under pressures of 30 MPa, temperature of 40°C and CO_2 flow rate of 2, 3, 4 ml/min. 5 g of powder sample of *S. mahagoni* seed was kept in the extraction vessel. The cotton is placed at the end to avoid any possible residue of solid material. The vessel is placed in an oven to maintain operating temperature. The extraction yield is collected in vial and placed in an oven to allow for evaporation solvent. And then, the extract is weighted and calculation of concentration yield is done based on cumulative mass of extract and stored at -20°C before further analysis for the extract yield and bioactive components.

2.3 Antioxidant Potential

2.3.1 Free Radical Scavenging Activity (DPPH)

The free radical scavenging of the *S. mahagoni* extracts was evaluated using the 1.1-diphenyl-2-picrylhydrazil (DPPH) method, as described [9] with a slight modification. Extract solution were prepared by dissolving 0.025 g of dry extract in 10 ml of methanol to give final concentration at 2.5 mg/ml. Then, 77 µL of the extract solution were mixed with 3 ml of 6 x 10^{-5} M methanolic solution of DPPH. After 30 min at room temperature, the absorbance values were measured at 517 nm in spectrophotometer. The DPPH radical concentration was calculated by using the following equation,

$$\text{DPPH radical concentration (\%)} = \frac{\text{A Control} - \text{A Sample}}{\text{A Control}} \times 100$$

where $A_{Control}$ is the absorbance value of the control reaction and A_{Sample} is the absorbance value with the presence of the tested extracts in the sample.

2.3.2 Total Phenolic Content (TPC)

The TPC was determined according to the Folin-Ciocalteau method [10] with slight modification. Briefly, the reaction mixture was composed by 1 mL of the extract (concentration of 0.01 g/mL), 5 mL of Folin-Ciocalteu reagent and 4 mL of sodium carbonate (75 g/L) and was allowed for 1 hour in the dark at room temperature. The absorbance was measured at 765 nm against a reagent blank (containing all test reagents except for sample). The TPC was calculated according to a standard curve. The concentration of total phenolic compounds in the extract was expressed as milligrams of Gallic acid equivalent per gram of dry weight (mg GAE/g) of extract. The content of phenolic compounds in the plant extracts were calculated using the following formula: C = A / B, Where C is expressed as mgGAE/g dry weight of the extract, A is the equivalent concentration of Gallic acid established from calibration curve (mg), and B is the dry weight of the extract (g) [10].

2.4 Antimicrobial Activity

The agar diffusion method Murray *et al.* [11] were used to evaluate the antimicrobial activity of the subjected extracts. Inoculum of 100 µL suspension containing 10^8 CFU/mL of bacteria was spread on Mueller Hinton Agar. The discs (9 mm in diameter) impregnated with 20 µL of 100 mg/mL extracts were placed on seeded agar medium. Streptomycin (10µg/disc) was used as positive control for bacteria. After that, the experiments were conducted in triplicate and the test plates were incubated 24 hours at 37° C for bacteria. Then, the diameters of zone of inhibition measured in mm [12].

2.5 Statistical Analysis

The data presented were analyzed by using SPSS 16.00 for Windows (SPSS Inc. Chicago, IL). Values were as mean ± standard deviation with three independent experiments. One-way analysis of variance (ANOVA) using Tukey's test at 95% confidence level were used to determine the significance difference between the samples.

■3.0 RESULTS AND DISCUSSION

3.1 Percentage of Extraction Yield by SC-CO_2

The yield of *S. mahagoni* seed extract is given in Figure 1. The extractive value indicates CO_2 flow rate 4 mL/min gives the maximum yield (19.67 %) amongst the other extracts. On the other hand, 2 mL/min flow rate CO_2 resulted in lowest extraction yield.

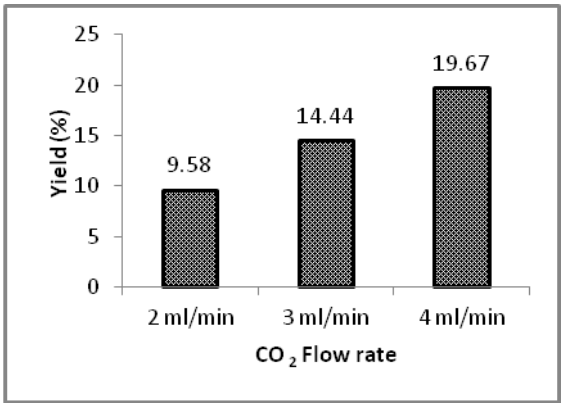


Figure 1 Percentage of extraction yield on different CO_2 flow rate

The extracted *S.mahagoni* oil yield increased from 9.58% to 19.67% with an increase in flow rate from 2 to 4 mL/min at pressure 30 MPa and temperature 40°C. The effect of flow rate was also studied for the extraction of hazelnut oil [13]. At a low pressure (15 MPa), an increase in flow rate from 0.5 to 2 ml/min (measured at extractor pressure and 10°C) did not cause a significant difference in the extraction yield of hazelnut oil. However, at high pressure (30 MPa), the oil yield increased more than three-fold [13].

3.2 Free Radical Scavenging Activity (DPPH)

The presented Table 1 shows the DPPH free radical scavenging assay result of three different carbon dioxide flow rate. The CO_2

flow rate 2 ml/min (94.40±5.38) gives highest DPPH radical scavenging activity compared to 3 mL/min (91.13± 4.84) and 4 mL/min (90.38± 1.53). This indicates the antioxidant ability of CO_2 flow rate 2 ml/min oils is stronger than the other two oils. The radical scavenging activity of the extracts could be related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating ability. Methanolic extract of the seed of *S. mahagoni* contain phenol and flavonoid which give antioxidant activity in vitro. The extract has potent antioxidant activity against free radical scavenging activity (DPPH) assays [14]. To date, the antioxidant activity of *S.mahagoni* seed extracts using types of CO_2 flow rate has not been well documented but from the results obtained, types of CO_2 flow rate does not give significant differences towards the scavenging of free radicals ($p>0.05$).

Table 1 DPPH free radical scavenging and TPC of *S. mahagoni* seed extract of different CO_2 flow rate

CO_2 Flow rate (mL/min)*	DPPH	TPC
2	94.40±5.38	72.75± 8.83
3	91.13± 4.84	67.98± 7.84
4	90.38± 1.53	62.20± 1.65

*Temperature 40°C; Pressure 30 MPa, value are mean ± standard deviation (n=3)

3.3 Total Phenolic Content (TPC)

Total phenolic content, as determined by the Folin-Ciocalteu method, is reported as gallic acid equivalents by reference to standard curve ($y = 0.00064x - 0.03186$) and $r^2 = 0.986$). The total phenolic content extract is shown in Table 1. As can be seen from Table 1, CO_2 flow rate 2 mL/min have higher phenolic content (72.75±8.83 mg GAE/g sample) compared to other CO_2 flow rate 3 mL/min (67.98±7.84 mg GAE/g sample) and 4 mL/min (62.20±1.65 mg GAE/g sample). That may be responsible for the antioxidative activities of this extract. Phenols and polyphenolic compounds, such as flavonoid, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities [10]. Different types of phenolic compounds have different antioxidant activity, which mainly depends on their structure as extract contains different types of phenolic compounds which have different antioxidant capacities. The statistical analysis indicated that types of CO_2 flow rate does not give significant differences towards the total phenolic content ($p>0.05$). In this study, obtained the positive linier correlation between antioxidant activity and total phenolic content.

3.4 Antimicrobial Activity

The antibacterial activity of *S.mahagoni* seed extracts using different CO_2 flow rate was studied by using disc diffusion method and the result are summarized in Table 2. This method involved the measurement of the inhibition zone by selected microorganism after 24 hours incubation.

Table 2 Antimicrobial activity for *S. mahagoni* seed extract of different CO_2 flow rate

CO_2 Flow rate (mL/min)*	*E.coli*	*B.Subtilis*	*S.aureus*
2	9.5±0.71[a]	8.5±2.12[a]	9.5±0.71[a]
3	13.5±2.12[a]	12.0±1.41[a]	11.5±0.71[a]
4	12.5±2.12[a]	9.0±2.83[a]	12.5±2.12[a]
Streptomycin	15.5±0.71[a]	15.0±0.00[a]	13.0±1.41[a]

*Temperature 40°C; Pressure 30 MPa, value are mean ± standard deviation (n=3)

As can be seen from Table 2, all extracts showed inhibitory effects which were not significantly different ($p>0.05$) against each bacterial species tested. In other words, the size inhibitory zones showed by all CO_2 flow rate did not differ significantly against all tested bacteria. After 24 hours, that CO_2 flow rate 3 mL/min extract was found to be active against the bacteria like *Escherchia coli* (13.5±2.12) and *Bacillus subtilis* (12.0±1.41). The results of disc diffusion assay of the crude extract were compared with that standart antibiotic Streptomycin (10µg/disc). Results also proved that CO_2 flow rate 3 mL/min extract has more effectiveness than that of CO_2 flow rate 2 mL/min and CO_2 flow rate 4 mL/min extract against subjected bacteria strains.

■4.0 CONCLUSION

Supercritical CO_2 extraction of *S. mahagoni* seeds was carried out and effects of CO_2 flow rate on the yields were studied. The yield of *S. mahagoni* seeds extract contained total phenolic compounds and were capable of inhibiting, quenching free radicals to terminate the free radical chain reaction, and acting as reducing agents. In the present study, a linear relationship was found between the antioxidant activity and phenolic content, indicating that phenolic compound could be major contributors to antioxidant activity. So it can be concluded that the seeds of *S. mahagoni* possess significant antimicrobial activity in terms of antibacterial properties.

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